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RESEARCH ARTICLE

Acridone-based acetylcholinesterase inhibitors; synthesis, antioxidant activity and molecular modeling

Heba Abd-Elhady El-gizawy[1], Farghaly Abdelhamid Omar[2] and Mohammed Abdalla Hussein[3*]

Abstract

Acridone is a unique naturally occurring alkaloid known to associate with several biological activities. 2,3-dimethoxy-10-methyl-10, 8a-dihydroacridin-9 (8aH)-one (**4)** and its precursor 2- ((3,4-dimethoxyphenyl)methylamino)benzoic acid (**3**) were synthesized and investigated for potential antioxidant and inhibitory activity against acetylcholinestrase. The synthetic pathway involves reaction of 2-(methylamino) benzoic acid (**1**) with 4-chloro-1,2-dimethoxybenzene (**2**) in presence of CuO and K_2CO_3 to give the precursor **3**. Subsequent, cyclcondensation of **3** with Conc. H_2SO_4 afforded the anticipated acridone **4**. Furthermore, the dimethoxyacridone derivative **4** showed potent antiacetylcholinesterase (ACHE) activity at **(**100 *uM*) with IC_{50} = 9.25 *uM* that is as potent as the reference drug rivastigmine. Assessment of total antioxidant activity of compounds **3 & 4** in comparison to known standard compounds revealed the following order: α-tocopherol > Acridone **4** > trolox > butylated hydroxyl anisole (BHA) > butylated hydroxyl toluene (BHT) > compound **3**. Molecular docking characteristics of **3 & 4** within the active site of AChE (PDB: 1ACJ) co-crystallized with 9-amino-tetrahydroacridine (Tacrine) have been studied. Interestingly, the results revealed comparable binding poses to the co-crystallized ligand and demonstrates good correlation of the binding energy (DG) with the observed IC_{50}-values. This finding suggests that compounds **3 & 4** exhibit good antioxidant effect and inhibition of acetylcholinesterase, which might provide profitable candidates in management of Alzheimer's disease.

Keywords: Acridone; antiacetylcholinesterase; antioxidant; docking; Alzheimer's disease; Molecular Docking

Introduction

Acridone alkaloids are biologically active fused heterocyclic rings. They are characteristic metabolites of plants belonging to the order Sapindales; more specific to Family Rutaceae. This Family commonly known as Rue or Citrus family; it is a Family of flowering plants with approximately 160 genera and over 2000 species. Several pharmacologically active constituents of plants belonging to this family have been identified; coumarin, furoquinoline, phenylpropanoids and acridone alkaloids which adds a specialty for this Family [1].

Acridone is unique alkaloid known to associate with several biological activities [2]. It has carbonyl group at 9^{th} position and nitrogen at 10^{th} position [3]. It is the oxidized product of acridine [4, 5]. Acridone is also known by the name of 9(10H)-acridinone, acridin-9-one, 9-acridanone, acridine-9 and 9-azanthracene-10-one [6]. The literature demonstrates that acridone shows anticancer [7, 8] , antimalarial [9], anti-inflammatory [10] , antiviral [11] and antibacterial activities [12].

As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds [13, 14];

*Correspondence: Prof.husseinma@o6u.edu.eg

[3]Biochemistry Department,, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, 12585, Egypt

Full list of author information is available at the end of the article.

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this paper reports the synthesis of two new compounds from a naturally occurring acridone alkaloid with the investigation of their antioxidant and AChEI activities of novel 2-((3,4-dimethoxyphenyl)methylamino)benzoic acid **3** and 2,3-dimethoxy-10-methyl-10,8a-dihydroacridin-9(8aH)-one **4**. As we know that acetylcholinesterase increases the amyloid protein fibrils formation which is toxic and linked to alzheimer's disease [15, 16]. Much physiology of acetylcholinesterase enzyme is not so far elucidated very well, so this is an interesting research area [17].

Latterly, in view of the efficiency of triazole derivatives in the inhibition of AChE [18], We reported the synthesis and in vitro evaluation of acridone linked to 1,2,3-triazoles [19]. Finally, molecular docking of the synthesized compounds to the active site of acetylcholinestrase will be also undertaken to delineate the underlying mechanism of the observed biological activity

Experimental

Chemistry

Melting points, infrared (IR), ^{1}H-, ^{13}C-NMR and Mass spectra were determined using Gallenkamp melting point apparatus, Shimadzu MR 470 infrared spectrophotometer, Varian EM 360 (^{1}H-NMR at 240MHz) & (^{13}C-NMR at 75MHz) and HP Model MS-5988, respectively. Elemental analytical data (C, H, N) were determined at the Microanalytical Center, Cairo University, Egypt.

2-(methylamino) benzoic acid, 4-chloro-1,2-dimethoxybenzene, CuO, K_2CO_3, α- tocopherol, (BHT), (BHA) and trolox were purchased from Sigma, USA. Ferrozine: a particular ferroin that is used for detection of iron. Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S Sigma-Aldrich). Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, and sodium hydrogen carbonate were purchased from Fluka.

Synthesis of 2-((3,4-dimethoxyphenyl)methylamino)benzoic acid (3)

To a solution of 2-(methylamino)-benzoic acid (9.66 g, 0.06 mol) and 4-chloro-1,2-dimethoxy-benzene (8.31 g, 0.06 mol) in mixture of anhydrous potassium carbonate and 3 g of copper oxide in 75 ml ethanol was prepared in a 250 ml round-bottomed flask fitted with an air-condenser. The mixture was boiled under reflux for 4-5 h. The mixture tended to foam during the earlier part of the heating owing to the evolution of carbon dioxide, and hence the large flask is used. When the heating was completed, the flask was fitted with a steam-distillation head and the crude product was steam-distilled until all the excess aniline has been removed. The residual solution contained the potassium N-phenylanthranilate. Two grams of animal charcoal was added to this solution and boiled for ~ 5 min, then filtered while hot. Diluted hydrochloric acid (1:1 v/v) was added to the filtrate until no further precipitation observed, and then the mixture was cooled in ice water with stirring. 2-((3,4-dimethoxyphenyl)methylamino)benzoic acid was filtered under reduced pressure, washed with water, drain and dried. The acid was recrystallized from aqueous ethanol with addition of charcoal [20]. Yield: 14.1 g(83%); m.p. 133-135°C; IR (KBr, cm^{-1}) : 3429 cm^{-1} (OH), 2989 cm^{-1} (CH-arom.), 2850 cm^{-1} (CH-aliph.), 1737(C=O). ^{1}H-NMR (DMSO-d_6) 3.5 [s, 6H, OCH_3], 2.5 (s, 3H, N-CH_3), 7.0–8.0 [m, 7H, Ar–H], 10.9 [s, 1H, COOH].^{13}C-NMR(75MHz, CD_3OD):148.2(C-1), 138.3(C-2), 116.3(C-3), 123.8(C-4), 142.3(C-5), 103.4(C-6), 40.2(C-7), 144.5(C-8), 113.1(C-9), 130.6(C-10), 118.4(C-11), 134.8(C-12), 120.4(C-13), 161.4(C-14), 56.6(C-15), 56.2(C-16). MS (m/z): 287 (M^+, 70.15%), 256 (5.00%), 225 (14.29%), 143 (81.00%), 114 (5.00%), 97 (100%). Elemental analysis, calc. for $C_{16}H_{17}NO_4$ (**287**); calc.(found): C: 66.89(66.15); H: 5.9(5.30); N: 4.80(5.20).

Synthesis of 2,3-dimethoxy-10-methyl-10,8a-dihydroacridin-9(8aH)-one (4)

A mixture of 5.74 g (0.02 M) of 2-((3,4-dimethoxyphenyl) methyl amino)benzoic acid (**3**) and 10 ml of conc. sulfuric acid was prepared in a conical flask and heated for 4 h on a steam bath. The hot dark green solution was poured slowly and cautiously into 200 ml of boiling water in a 500 ml beaker, allowed the acid to run down the side of the beaker to avoid "spattering". The mixture was boiled for 5 min, and it was filtered while hot through a Buchner funnel. The precipitate was washed with hot water. For purification, acridone was transferred to a solution of 4 g of hydrated sodium carbonate in 50 ml of water, boiled for 5 min, and then filtered while hot. Acridone was washed with boiling water and dried thoroughly. Recrystallization from acetic acid using charcoal or better sublimation, gives the bright yellow product. Yield 10.18 g (70%); m.p. 310-312°C. IR (KBr, cm^{-1}): 3041 and 2927 cm^{-1} (CH-arom.), 2854 cm^{-1} (CH-aliph.), 1542 (C=O), 1504 (-CH_2- def.),1256, 1130 (C-CO-C, diaryl ketone), 1095 (C-O), 879 (1,2,4-trisubstituted benzene),. ^{1}H-NMR (DMSO-d6): 2.3 (s, 3H, N-CH_3), 2.9 [s, 6H, OCH_3], 7.2–7.7 [m, 6H, Ar–H]. ^{13}CNMR(75MHz, CD_3OD): 118.4(C-1), 112.2(C-1′), 138.3(C-2), 147.7(C-2′), 148.2(C-3), 98.6(C-4), 129.7 (C-5), 49.9 (C-5′), 123.8(C-6), 130.6(C-7), 134.8(C-8), 50.9(C-8′), 190.0(C-9), 36.5(C-10), 56.2(C-11), 59.0(C-12). MS (m/z): 271 (M^+, 36%), 238 (51%), 190 (73%), 143 (78%), 104 (86%),

90 (100%). Elemental analysis, calc. for $C_{16}H_{12}NO_3$ (**271**); calc.(found): C: 70.84(71.00); H: 4.42(4.80); N: 5.16(5.20).

Biological testing

Estimation of AChE-Inhibitory Activity, *in-vitro*

Assessment of IC_{50} of ACHE inhibitory activity has been carried out according to Zdrazilova's modification [21]. Compounds **3** and **4** were dissolved in 10 ml of a mixture of DMSO-methanol (1:1) and diluted with 0.1 *M* KH_2PO_4/K_2HPO_4 buffer (pH = 8.0) to obtain final concentrations of 25, 50 and 100 μM. All experiments were achieved at 25 $^\circ C$, using three different concentrations for each compound and tested in triplicate to attain a range of 20–80% inhibition of AChE. The modified procedure involves using a 96-well plate reader (BioTek ELx808, Highland Park, IL, USA). Each well contain 50 *ul* potassium phosphate buffer (KH_2PO_4/ K_2HPO_4, 0.1 *M*, pH= 8), the respective amount of sample (25, 50 and 100 *ul* dissolved in DMSO-methanol mixture, and 25 *ul* enzyme (final concentration 0.22 *U/ml* in buffer). They were incubated for 20 *min* at room temperature, and then, 125 *ul* of 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) (3 *mM* in buffer) was added. The hydrolysis of acetythiocholine, catalyzed by AChE, after 20 min of addition then determined by measuring the change in absorbance spectrophotometrically at λ_{max} = 405 *nm.* The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration versus percent of inhibition). A control experiment was performed under the same conditions without inhibitor and the blank contained buffer, DMSO, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), and substrate.

Antioxidant properties of synthesized compounds 3 and 4

Evaluation of reducing power

The reducing power of compounds **3** and **4** was measured according to the method of Oyaizu [22]. The absorbance was measured at λ_{max} =700 nm. α- tocopherol, BHA and BHT were used as standard antioxidants.

Estimation of free radical scavenging activity

The free radical scavenging activity of compounds **3** and **4** was measured with 1,1-diphenyl-2-picrylhydrazil ($DPPH^{\cdot}$) using the modified method of Takashira and Ohtake [23] . The percent inhibition activity was calculated using the following equation:

(%) Inhibition = $[(A_0 - A_1)/A_0 \times 100]$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds **3** and **4** samples.

Evaluation of total antioxidant activity

The total antioxidant activity was evaluated according to the thiocyanate method of Osawa and Namiki [24] with slight modifications. α- tocopherol, trolox, BHA and BHT were used as standard antioxidants.

The % inhibition of lipid peroxidation was calculated according to the following equation:

% Inhibition = $[(A_0 - A_1)/A_0 \times 100]$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds **3** and **4** or standards.

Detection of chelating activity on Fe^{2+}

The chelating activity of compounds **3** and **4** with ferrous ions (Fe^{2+}) was detected according to the method of Decker and Welch [25]. The absorbance was measured spectrophotometrically at λ_{max} = 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of compounds **3** and **4** were compared with that of EDTA at a level of 0.037 *mg/ml*.

% Chelating activity was calculated using the following formula:

[1 – (Absorbance of sample / Absorbance of control)] x 100]

Control test was performed without addition of compounds **3** and **4**

Molecular docking

Docking simulation study has been performed in the CADD Lab. (Dept. of pharmaceutical Chemistry; Faculty of Pharmacy; Oct. 6 University) using Molecular Operating Environment (MOE®) version 2014.09, Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory.

Target compounds optimization The target compounds were constructed into a 3D model using the builder interface of the MOE program. After checking their structures and the formal charges on atoms by 2D depiction, the following steps were carried out:

- The target compounds were subjected to a conformational search.
- All conformers were subjected to energy minimization, all the minimizations were performed with MOE until a root mean square deviation (RMSD) gradient of 0.01 Kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated.

- The obtained database was then saved as Molecular Data Base (MDB) file to be used in the docking calculations.

Optimization of the enzymes active site The X-ray crystallographic structure of acetylcholinesterae (PDB Code: 1ACJ), co-crystallized with tacrine, was obtained from RCSB-Protein data bank. The enzyme was prepared for docking studies by:

- Hydrogen atoms were added to the system with their standard geometry.
- The atoms connection and type were checked for any errors with automatic correction.
- Selection of the receptor and its atoms potential were fixed.
- MOE Site Finder was used for the active site search in the enzyme structure using all default items. Dummy atoms were created from the obtained alpha Spheres.

Docking of the target molecules to Acetylcholinesterase active site Docking of the conformation database of the target compounds was done using MOE-Dock software. The following methodology was generally applied:

- The enzyme active site file was loaded and the Dock tool was initiated. The program specifications were adjusted to:

- Ligand atoms as the docking site.

- Triangle matcher as the placement methodology to be used.

- London dG as Scoring methodology to be used and was adjusted to its default values.

- The MDB file of the ligand to be docked was loaded and Dock calculations were run automatically. The obtained poses were studied and the poses showed best ligand-enzyme interactions were selected and stored for energy calculations.

Statistical analysis

All the grouped data were statistically evaluated with SPSS/18 software. Hypothesis testing methods included one way analysis of variance (ANOVA). *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for three successive measurements.

Results and Discussion

Chemistry

The synthesis pathway for preparation of 2-((3,4-dimethoxyphenyl) methyl amino) benzoic acid **3** and 2,3- dimethoxy-10-methyl-10, 8a-dihydroacridin-9 (8aH)-one **4** is illustrated in Scheme 1.

The structures of compounds **3** and **4** were ascertained on basis of elemental analyses, IR, mass, ^{1}HNMR and ^{13}CNMR spectral data. The IR spectrum of compound **3** showed bands at 3429 cm^{-1} (OH), 2989 cm^{-1} (CH-arom.), 2850 cm^{-1} (CH-aliph.), 1737(C=O). Its MS exhibited molecular ion peak 287 (M^{+}, 70.15%) with base peak at 97 (100%) in addition to other significant peaks at 225 (14.29%), 143 (81.00%) and 114 (5.00%). The ^{1}H-NMR spectrum in DMSO-d_6 shows signals at δ: 2.5 (s, 3H, N-CH_3), 3.5 [s, 6H, OCH_3], 7.0–8.0 [m, 7H, Ar–H], and 10.9 [s, 1H, COOH]. The most characteristic signals in ^{13}C-NMR spectrum of compound **3** in deuterated methanol (CD_3OD) due to the methoxy and N-methyl groups appeared at δ = 56.6, 56.2, and 40.2 ppm respectively. Moreover, the spectrum showed the signals of the aromatic C-atoms at the expected δ-values and that of the carboxylic (C=O) at 161.4 ppm.

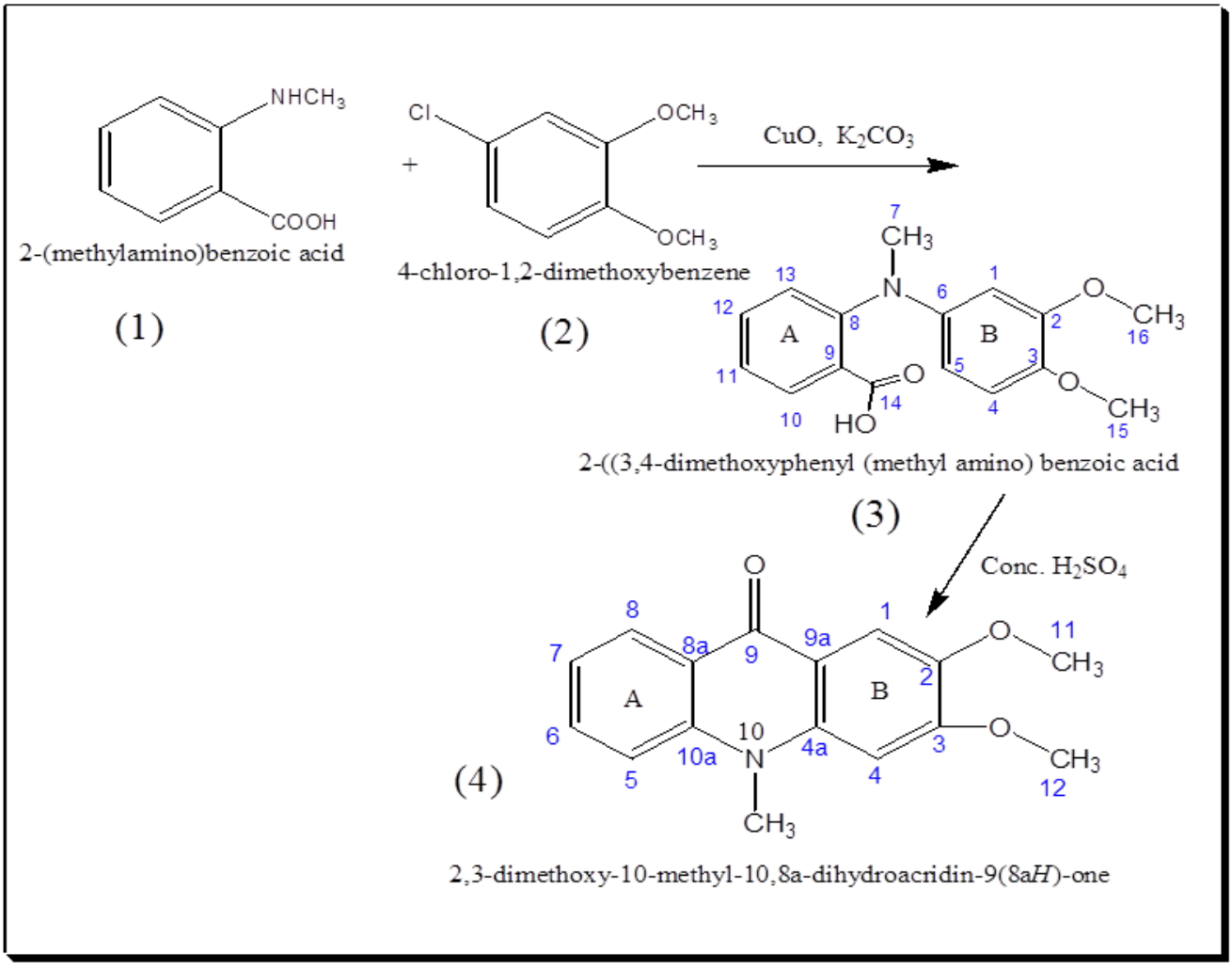
The formation of compound **4** is assumed to proceed via dehydration of the two molecules of the acid (3) to intermediate acid hydride followed by cyclization involving nucleophilic addition and elimination of water to give the acridone **4**, (Scheme 2). The IR spectrum of compound **4** showed bands at 3041 and 2927 cm^{-1} (CH-arom.), 2854 cm^{-1} (CH-aliph.), 1542 cm^{-1} (C=O), 1504.7 cm^{-1} (-CH_2- def.), 1256.7, 1130.7 cm^{-1} (C-CO-C, diaryl ketone), 1095.8 cm^{-1} (C-O), 879.8 cm^{-1} (1,2,4-trisubstituted benzene). In MS The molecular ion peak 271 (M^{+}, 36%) with base peak at 90 (100%) and other significant peaks at 238 (51%), 190 (73%), 143 (78%) and 104 (86%) are displayed in its mass spectrum. ^{1}H-NMR spectrum (DMSO-d_6) exhibited signals at 2.3 (s, 3H, N-CH_3), 2.9 [s, 6H, OCH_3], 7.2–7.7 [m, 6H, Ar–H]. ^{13}C-NMR spectrum in (CD_3OD) exhibited signals at, 36.5(N-**C**H_3), 56.2(OCH_3), 59.0(OCH_3), 190.0(C=O) indicating the formation of acridone ring.

Biological activity

AChE-Inhibitory Activity

The IC_{50} values of compounds **3** and **4** were determined using the spectrophotometric Ellman's method [26], which is a simple, rapid and direct method originally developed to determine the SH and -S-S- group content in proteins [27]. The method is widely applied for assessment of the activity of cholinesterase inhibitors. Cholinesterase activity is measured indirectly by quantifying the concentration of 5-thio-2-nitrobenzoic acid (TNB) ion formed in the reaction between 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and thiocholine. The latter is produced from hydrolysis of acetylthiocholine (ACh) by cholinesterase [28].

The IC_{50} values of the studied compounds **3**, **4** and rivastigmine (Exelon®), a known ACHEI, as a reference drug are summarized in Table 1. Both compounds exhibited slightly lower



Scheme 1: Synthetic pathway of the targeted compounds 3 & 4

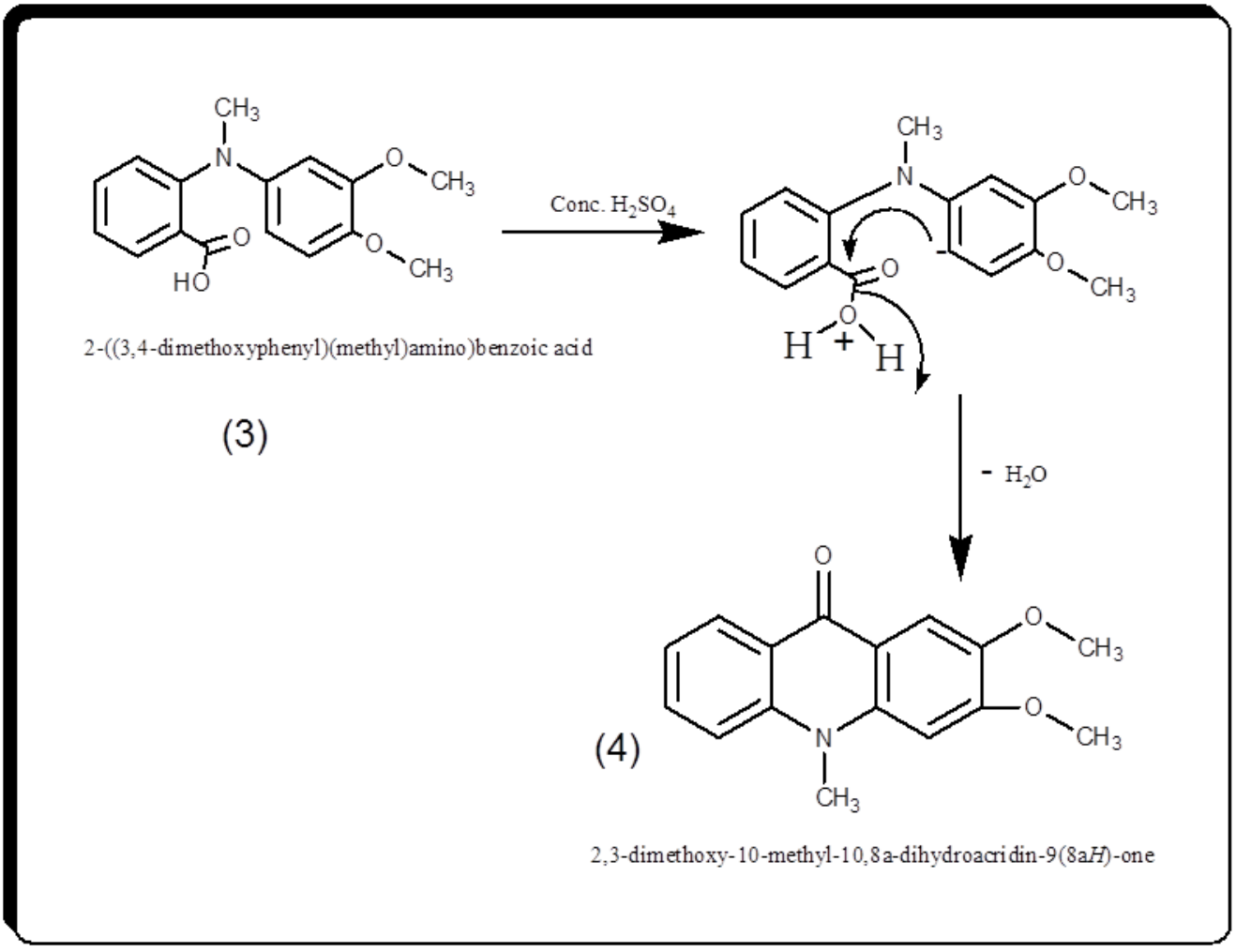
inhibitory activity than the standard drug. Interestingly, compound **3** showed higher anticholinesterase activity than the cyclized rigid analog **4** at the studied concentrations of 25, 50, and 100 μM, and comparable or slightly higher inhibitory activity than rivastigmine at 50 and 100 μM respectively. This 2-(3,4-dimethoxypheny-methylamino) benzoic acid **3** is a flexible molecule and can attain different conformational orientations within the active site of the enzyme, that might be considered as carbamate-like substrate.

Table 1 The IC_{50} values of the compounds 3, 4 and rivastigmine against acetylcholinesterase (AChE).

Compds. #	Conc.(μM)	AChE inhibition IC50 (μM)
3	25	33.5±0.08
	50	16.7±0.04
	100	9.25±0.08
4	25	>100
	50	>100
	100	>100
Rivastigmine	25	11.55

Antioxidant activity

The antioxidant potential of the studied 2-(3, 4-(dimethoxyphenyl)-methylamino) benzoic acid **3** and the cyclized acridone analog **4** were investigated through assessment of antioxidant potential. Figure (**1**) shows the results expressed as the absorbance of the formed Perl's Prussian blue at λ_{max} = 700 nm, formed by the effect of compounds **3, 4** in comparison to standard antioxidants BHA, BHT and α-tocopherol. The maximum reducing activity could be attained at ~120 $\mu g/ml$. This indicates that compounds **3** and **4** are electron donors and could react with free radicals, converting them to more stable products and terminate the radical chain reaction. The reducing power of compound **4** was pronounced than BHA, BHT and compound **3**.



Scheme 2: Proposed formation of compound 4

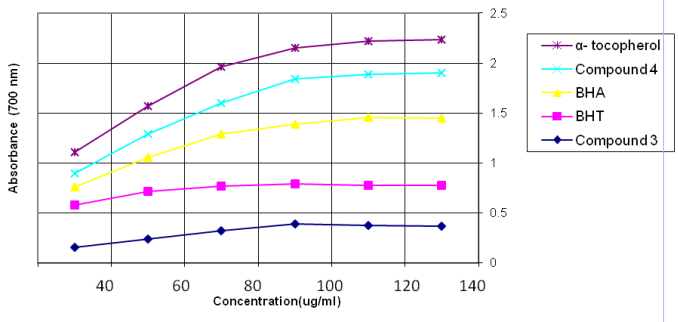


Figure 1 Reducing power of compounds 3 and 4, BHA, BHTand α-tocopherol

1,1-Diphenyl-2-picrylhydrazil (DPPH$^{\bullet}$) is used as a free radical to evaluate the antioxidative activity of some naturally occurring compounds [14, 29] . The DPPH$^{\cdot}$ radical scavenging effects of compounds **3** and **4** compared to trolox, as a synthetic antioxidant are presented in Fig. **(2)**, illustrating appreciable free radical scavenging activities. The studied compounds **3** and **4** showed maximum radical scavenging activity at 6 *mg/ml* compared with 0.06 mg/ml for Trolox.

The antioxidant activity of compound **4** (four resonating structures) is more pronounced than compound **3** (two resonating structures), (**scheme 3**), due to the presence of conjugated double bond system, which makes the electrons more delocalized to the carbonyl group [30]. The antioxidant activity of phenolic compounds may result from the neutralization of free radicals [31, 32]. It is also known that the antioxidant activity of phenolic compounds is closely associated with their structures, such as substitutions on the aromatic ring and side chain structure [33]. The structural requirement considered essential for effective radical scavenging by the acridone derivative **4** is the presence of methoxy groups in B ring and conjugation to the C=O.

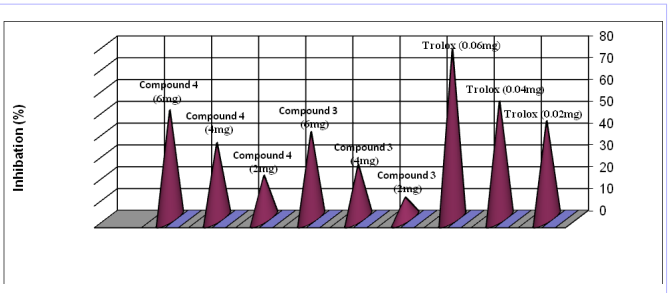


Figure 2 Scavenging activities of different concentrations of compounds 3 and 4 and trolox against the1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) radical.

The total antioxidant potential expressed as the inhibitory effect on peroxidation of linoleic acid emulsion at 100 *mg/l* of compounds **3, 4** and standard antioxidants are shown in Fig. **3**. In this respect compound **4** showed higher antioxidant activity than trolox, BHA, and BHT. The total antioxidant activity decreased in the order: α-tocopherol > compound **4 >** Trolox > BHA > compound **3** > BHT.

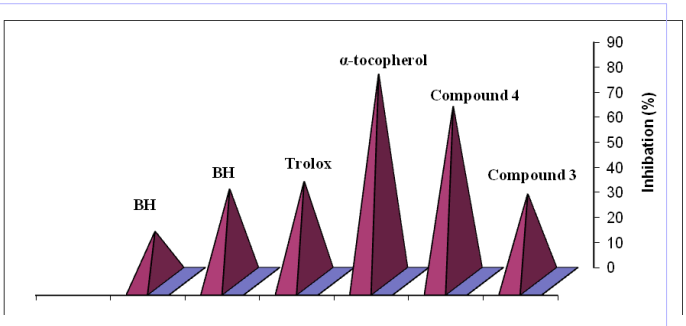


Figure 3 Total antioxidant activities of compounds 3 and 4, α-tocopherol, Trolox and BHA, BHT (100 mg/l concentration) on peroxidation of linoleic acid emulsion

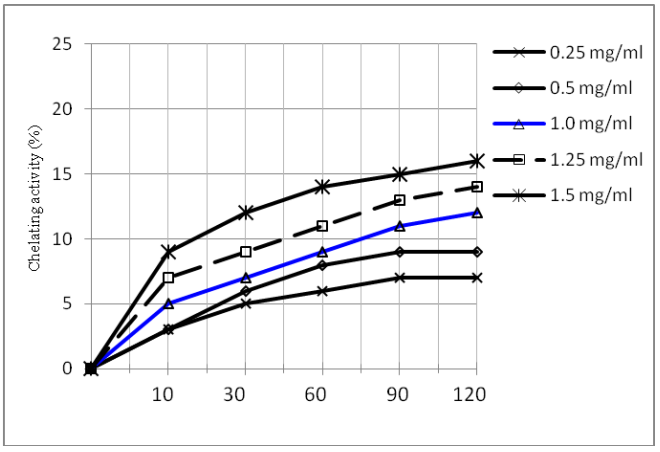


Figure 4 Chelating effect of different concentrations of compound 3 on Fe^{2+} ions ($FeCl_2$) at different incubation times.

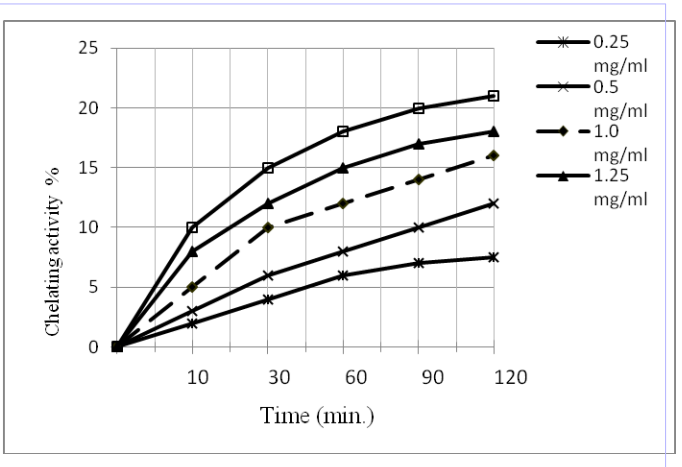


Figure 5 Chelating effects of different concentrations of compound 4 on Fe^{2+} ions ($FeCl_2$) at different incubation times.

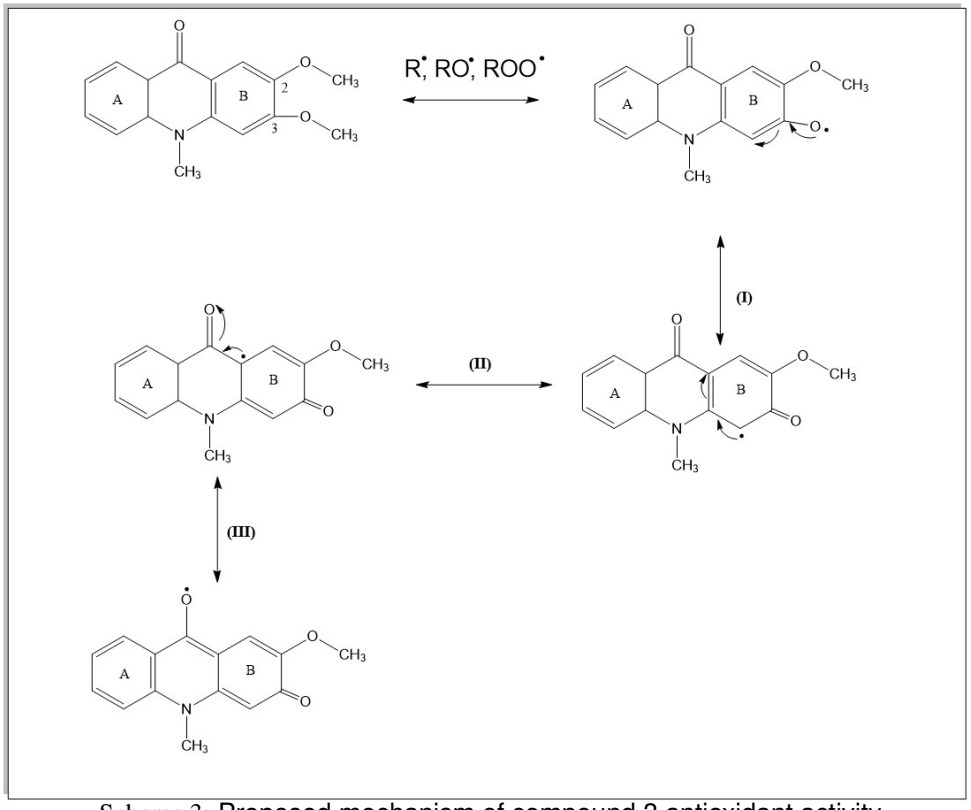
Iron (Fe^{2+}) chelating activity

The chelating effect of compounds **3** and **4** on ferrous ions has been accomplished and the results are showed in **Figures 4a, b** respectively. All samples showed the best chelating effect of 16% and 21% at concentration = 1.5 *mg/ml* after an incubation time of 60 min. The results in **Fig. 4a, b** indicated that a significant property of compounds **3** and **4** is their capability of blocking the oxidative activity of systems with transition metal ion (Fe^{2+}/Fe^{3+}) that play an essential role in the formation of reactive oxygen species in Fenton's reactions.

Molecular docking Study

Molecular docking within the present investigation was carried out using Molecular Operating Environment (MOE®) version 2014.09, to explore the possible ligand-target interactions that might explain the inhibitory effect of the synthesized compounds on acetylcholinesterase. The study involves 2,3-dimethoxy-10-methyl-10,8a-dihydro-acridin -9(8a)-one **(4)**, as bioisosteric analogue of tacrine, in addition to its precursor, 2-(N-(3,4-dimethoxyphenyl)-methylamino) benzoic acid **(3)**, as flexible analogue.

Structural analysis of AChE revealed the active site placed in a cavity near the bottom of a narrow imbedded halfway into the protein, with 14 aromatic amino acid residues lining a substantial portion of its surface [34]. This is named as the 'active site gorge' and, further at the gorge mouth a peripheral anionic binding site (PAS) was found. All 14 amino acids in the aromatic gorge are highly conserved across different species [35]. Among the aromatic amino acids, tryptophan 84 is critical and



Scheme 3: Proposed mechanism of compound 3 antioxidant activity

its substitution with alanine results in a 3000-fold decrease in reactivity [36]. The gorge penetrates halfway through the enzyme and is approximately 20 Å long [37].

The active site of AChE contains: (1) an esteratic site (ES) comprising the catalytic triad *Ser*200-*His*440-*Glu*327, which is located at the bottom of the gorge; (2) an oxyanion hole (OAH) that stabilizes the tetrahedral intermediate binding of the carbamate carbonyl group; (3) an acyl binding site (ABS) that binds the acetyl group of ACh or the alkyl moiety of carbamate inhibitors; (4) an anionic substrate binding site (AS) that contains a small number of negative charges but many aromatic residues, where the quaternary ammonium pole of ACh and of various ligands binds through a preferential interaction of quarternary nitrogens or a partial positive charge generated by electron-withdrawing moieties.

Acetylcholinesterae inhibitors are diverse including several natural and synthetic chemical classes. Carbamate inhibitors would be the most efficient [38, 39], meanwhile, other chemical classes are recognized and approved as ACHEIs inhibitors involving: tetrahydro-aminoacridine (THA') also known as Tacrine; Donepezil; galantamine etc. that has been widely used therapeutically for management of neurodegenerative conditions.

Several X-ray crystal structures of acetylcholinesterase are available online. Only some of them are co-crystallised with the tricyclic inhibitor, Tacrine [37]. Based on structural relevance of the studied compounds to Tacrine, the crystal structure of acetylcholinesterase (PDB code: 1ACJ) has been downloaded from Protein Data Bank (https://www.rcsb.org), prepared and used in the current molecular docking study. Analysis of the ligand-enzyme interaction (Fig. 6) revealed that the acridine nucleus is stacked against the indole of *Trp*84; the phenyl ring of *Phe*330 is positioned to make hydrophobic contact with the bound ligand, and the amino group displays bifurcated hydrogen bonding with two members of the catalytic triad, *Ser*81 and *Asp*72. The structural and chemical data, together, show the important role of aromatic groups as binding sites for ligands, and they provide complementary evidence assigning *Trp*84 *and Phe*330 to the "anionic" subsite of the active site.

In the present investigation the proposed docking algorithm was initially validated by re-docking of Tacrine, the co-crystallized ligand, to acetylcholinesterase PDB code: 1CAJ). Whereby, the ligand removed from the complex and then docked back into the binding site. Heavy-atom root mean square deviation (RMSD) values between top-ranked poses and the experimental crystal structure ranged from ~ 0.2639 to 0.5883 Å. Subsequently, docking procedures have been executed for the

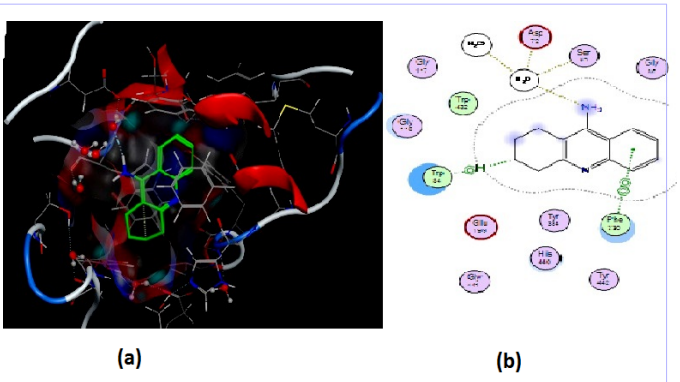


Figure 6 Surface map of Tacrine (green)co-crystallized with acetylcholinesterase (1ACJ) (a); and the corresponding interaction diagram (b).

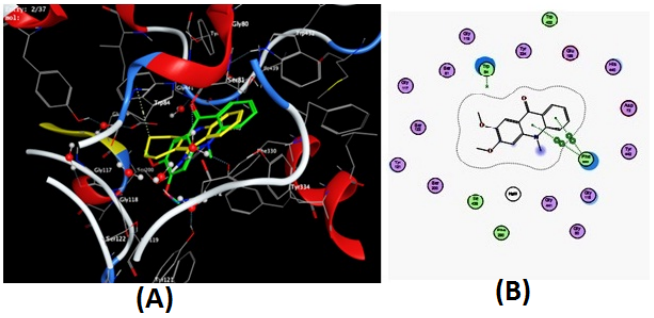


Figure 7 a) 3D representation of dimthoxyacridone derivative (green)and the cocrystallized ligand, tacrine(yellow); b) Ligand interaction diagram of dimethoxyacridone 4.

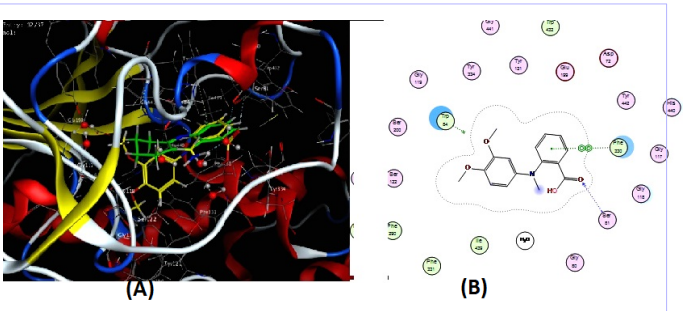


Figure 8 a) 3D representation of dimthoxybenzamide derivative(yellow) and the cocrystallizedligand, Tacrine (green); b) Ligand interaction diagram of 3.

investigated compounds **3 & 4**. The study successfully identified binding poses with comparable docking scores (**Table 2**), indicating that the compounds could potentially bind to the active site with analogous strengths.

The results in Table 2, showed that the investigated compounds displaying comparable binding ability to acetylcholinesterase, as evident by the observed docking scores (-7.4 – 7.1 *kcal/mol*) relative to that of the co-crystallized ligand (-6.04 *kcal/mol*). In addition, common interaction modes are also observable. Generally, the observed AChE-inhibiting behavior of the studied compounds **3** and **4** can be attributed to facilitated entrance to the gorge and slightly different fitting of (**3**) to the active site of AChE.

The deviation from the catalytic triad binding site can be explained as a result of the presence of the bulky methoxy groups as substituents. This is well illustrated in case of the rigid dimethoxyacridone derivative (**Fig. 7**), whereby its binding restricted to π- π interaction with the aromatic amino acid residue *Phe-330*. On the other hand, the flexible analog, dimethoxy-N-methylbenzamide binds to *Ser-81* in the catalytic triad position through H-bond in addition to the π- π interaction with *Phe-330* (**Fig. 8**). Such dual-binding site inhibitors of AChE , e.g. donepezil, were described by Kryger and Alonso [38, 40, 41] showing interaction with both the active and the peripheral sites and might accounts for long acting or what is known as pseudo-irreversible ACHE inhibition.

According to our results, compound **3** showed the highest ACHE inhibitory activity (IC_{50} = 9.25 *uM*), However, the activity greatly reduced as result of structural rigidification upon cycliztion to the corresponding acridone.

Conclusion

Novel dimethoxyacridone have been designed, synthesized, and evaluated as an efficient antioxidant and anti-AChE compounds. 2-(N-(3,4-dimethoxyphenyl)-methylamino)benzoic acid **(3)** (100 *uM*) showed the highest inhibitory activity of AChE (IC_{50} = 9.25 *uM*) being as potent as reference drug, rivastigmine. Insertion of dimethoxy moieties at 2, 3 position to acridone lead to higher potency as anti-AChE agent. Also, the present study showed that antioxidative activity of compound **4** was more pronounced than **3** depending on its resonating structures. More studies are needed to prove their medicinal and biological importance which may pave the way for possible therapeutic applications.

Author details

[1]Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Sixth of October City, 12585,, Egypt. [2]Department of Pharmaceutical Chemistry,, Faculty of Pharmacy, October 6 University, Sixth October City, 12585, Egypt. [3]Biochemistry

Table 2 Docking Scores &interaction modes of tacrine and the investigated Compounds 3 and 4

Compds.	**Docking Scores (-dG:Kcal/mol)**	Interaction	**Distance (Å)**	E(kcal/ mol)
Tacrine	-6.0433	H-donner:(-NH_2/H_2O-634/Ser81)	3.12	-1.2
			3.12	-1.2
		H-donner:(-NH_2/H_2O-634/Asp72)	3.96	-0.0
			4.18	-0.8
		π- π: (phenyl/Phe330)		
		H-π:(tetrahydrophenyl/ Trp84)		
Compound **3**	-7.3507	H-acceptor: COO-/Ser81	3.18	-0.8
		π- π: (phenyl/Phe330)	3.74	-0.0
Compound **4**	-7.1066	π- π: (phenyl/Phe330)	3.92	-0.0
		π- π: (Pyridone/Phe330)	3.99	-0.0

Department,, Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, 12585, Egypt.

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